



In the Specification:

Please amend the specification as shown:

Please delete the paragraph on page 3, lines 12-13, and replace it with the following paragraph:

C1
FIG. 2(a) provides the *papH* DNA sequence of pHUR849 (SEQ ID NO: 31), FIG. 2(b) pDAL201B *papH* (SEQ ID NO: 33), FIG. 2(c) pDAL210B *papH* (SEQ ID NO: 35), and FIG. 2(d) pDAL200A (SEQ ID NO: 37).

Please delete the paragraph on page 3, lines 14-15, and replace it with the following paragraph:

C2
FIG. 3 provides a comparison of the *papH* DNA sequences of pHUR849 (SEQ ID NO: 31), pDAL200A (SEQ ID NO: 37), pDAL201B (SEQ ID NO: 33), and pDAL210B (SEQ ID NO: 35).

Please delete the paragraph on page 3, lines 16-17, and replace it with the following paragraph:

C3
FIG. 4 gives a comparison (SEQ ID NO: 39) of deduced amino acid sequences of *papH* genes for pHUR849, pDAL200A, pDAL201B, and pDAL210B.

Please delete the paragraph on page 3, lines 18-20, and replace it with the following paragraph:

C4
FIG. 5(a) shows the amino acids (which are underlined) that are deleted from *papH* in pHUR849 (SEQ ID NO: 32) and FIG. 5(b) shows the amino acids (which are

underlined) that are deleted from papH in pDAL201B (SEQ ID NO: 34), pDAL210B (SEQ ID NO: 36), and pDAL200A (SEQ ID NO: 38).

Please delete the paragraph on page 7, lines 18-24, and replace it with the following paragraph:

The following peptide conjugate vaccines were demonstrated to be protective after serial parenteral administration in the BALB/c murine model of experimental pyelonephritis after intravesicular administration:

<u>F serotype</u>	<u>Structural Pilin Sequence</u>	<u>Residue Position (R)</u>
F13 (<u>SEQ ID NO: 1</u>)	PQGQGKVT	R 5-12
F13 (<u>SEQ ID NO: 2</u>)	AKFGGMGAKKG	R 65-65

Please delete the paragraph on page 8, lines 21-34, and replace it with the following paragraph:

The following peptide thyroglobulin and bovine serum albumin conjugate vaccines were made:

<u>F serotype</u>	<u>Structural Pilin Sequence</u>	<u>Residue Position (R)</u>
F71 (<u>SEQ ID NO: 3</u>)	PQGQGEVSF	R 5-12
F71 (<u>SEQ ID NO: 4</u>)	NFKQLQGGAACKG	R 65-77
F72 (<u>SEQ ID NO: 5</u>)	PQGQGKVTF	R 5-12
F72 (<u>SEQ ID NO: 6</u>)	NFKKAAGGGGAKT	R 65-75
F9 (<u>SEQ ID NO: 7</u>)	QSGSQVNFKG	R 4-12
F9 (<u>SEQ ID NO: 8</u>)	NFKKAATPGGAAKT	R 65-75
F11 (<u>SEQ ID NO: 9</u>)	IPQGQGKVTFNG	R 4-15
F12 (<u>SEQ ID NO: 10</u>)	IPEGQGKVT	R 2-12
F1C (<u>SEQ ID NO: 11</u>)	NGGTVHFKGEVVN	R5-12
F1 (<u>SEQ ID NO: 12</u>)	TTVTVNGGTVHF	R4-15

Please delete the paragraph on page 10, lines 5-18, and replace it with the following paragraph:

Results indicated the following:

<u>F serotype</u>	<u>Pilin A Sequence</u>	<u>Residue Positions</u>	<u>Homologous Protection</u>
C7 { F71 (SEQ ID NO: 13)	PQGQGEVT	R 5-12	Yes
F71 (SEQ ID NO: 14)	PQGQGEVA	R 5-12	Yes
F71 (SEQ ID NO: 4)	NFKQLQGGAACKG	R 65-77	Yes
F72 (SEQ ID NO: 5)	PQGQGKVT	R 5-12	Yes
F72 (SEQ ID NO: 6)	NFKKAAGGGGAKT	R 65-77	Yes
F9 (SEQ ID NO: 15)	TTVNGGTVH	R 4-12	Yes
F9 (SEQ ID NO: 8)	NFKKAATPGGAAKT	R 65-75	Yes
F11 (SEQ ID NO: 16)	IPQGQKVTFNQTV	R 4-17	Yes
F12 (SEQ ID NO: 10)	IPEGQGKVT	R 4-12	Yes
F1C (SEQ ID NO: 11)	NGGTVHFKGEVVN	R 5-15	Yes
F1 (SEQ ID NO: 12)	TTVTVNGGTVHF	R4-15	Yes

Please delete the paragraph on page 10, lines 20-41, and replace it with the following paragraph:

One or a combination of pilin A vaccines comprising one or more of the following amino acid sequences that correspond to published and unpublished F pilin primary sequences would be protective against ascending, non-obstructive *Escherichia coli* urinary tract infections in anatomically normal women and males:

		Pilin A Residue Urinary Tract		New or
<u>F serotype</u>	<u>Pilin A Sequence</u>	<u>Positions</u>	<u>Protection Potential</u>	<u>Old Claim</u>
4- F71 (SEQ ID NO: 13)	PQGQGEVT	R 5-12	Pyelonephritis	New
P71 (SEQ ID NO: 14)	PQGQGEVA	R 5-12	Pyelonephritis	New
C8 2- F71 (SEQ ID NO: 4)	NFKQLQGGAACKG	R65-77	Pyelonephritis	New
- F72 (SEQ ID NO: 5)	PQGQGKVT	R 5-12	Pyelonephritis	New
F72 (SEQ ID NO: 6)	NFKKAAGGGGAKT	R65-77	Pyelonephritis	New
3- F9 (SEQ ID NO: 15)	TTVNGGTVH	R 4-12	Pyelonephritis	New
F9 (SEQ ID NO: 8)	NFKKAATPGGAAKT	R 65-75	Pyelonephritis	New
2- F11 (SEQ ID NO: 16)	IPQGQGKVTFNGTV	R 4-17	Pyelonephritis	New
1- F12 (SEQ ID NO: 10)	IPEGQGKVT	R 4-12	Pyelonephritis	New
2- F13 (SEQ ID NO: 1)	PQGQGKVT	R 5-12	Pyelonephritis	Old
F13 (SEQ ID NO: 17)	AKFGGMGAKKG	R 65-65	Pyelonephritis	Old
1- F1C (SEQ ID NO: 11)	NGGTVHFKGEVVN	R 5-15	Cystitis	New
- F1 (SEQ ID NO: 12)	TTVTVNGGTVHF	R4-15	Cystitis	New

Please delete Table 2 on page 19 and replace it with the following Table:

TABLE 2. Primers used in this study

Primers	Oligonucleotide sequence	Description
T3	5' ATTAACCCTCACTAAAG 3' (SEQ ID NO: 18)	anneals to multiple cloning site of SK-
T7	5' AATACGACTCACTATAG 3' (SEQ ID NO: 19)	anneals to multiple cloning site of SK-
Reverse	5' AACAGCTATGACCATG 3' (SEQ ID NO: 20)	anneals to multiple cloning site of SK-
PGpHFD	5' ATGAGACTGCGATTCTCTGT 3' (SEQ ID NO: 21)	anneals to the TAC translational start region of all 4 <i>pap</i> H genes
PapHRE	5' TCCGTTTCTCACAATTCTGA 3' (SEQ ID NO: 22)	anneals to bp 509-528 of the <i>pap</i> H gene of pDAL201B, <i>pap</i> -21 and pHUR 849, <i>pap</i> -5
210bFD	5' CCTGAAATACGAGAATATTA 3' (SEQ ID NO: 23)	anneals 93-bp upstream of the TAC translational start region of the <i>pap</i> A gene of pHUR849, <i>pap</i> -5 (2)
210bRE	5' TAATATCTCGTATTTTCAGG 3' (SEQ ID NO: 24)	the complement of 210bFD and anneals to the same 93-bp region as described for 210bFD
FOR210b	5' TGGACTGGTATAACAATCGA 3' (SEQ ID NO: 25)	anneals 2.9 kb upstream of the TAC translational start region of the <i>pap</i> H gene of pDAL210B, <i>pap</i> -21
200aRE	5' TCCGTTTCGCACAATTCTGA 3' (SEQ ID NO: 26)	anneals to bp 511-528 of the <i>pap</i> H gene of pDAL210B, <i>pap</i> -17, and <i>pap</i> 200a, respectively
PapFOR ^a	5' AGTGGATTTCATGCAGCATTTCT AGAAA 3' (SEQ ID NO: 27)	anneals to bp 258-270 of the <i>pap</i> A gene of pHUR849, <i>pap</i> -5 (2)
FORSEQ	5' TGGACCTCCTGAGCTA 3' (SEQ ID NO: 28)	anneals to bp 456-474 of the <i>pap</i> A gene of pHUR849, <i>pap</i> -5 (2)
PapREV ^b	5' GGGGCAGCCCTGCCGTCCCAA AT 3' (SEQ ID NO: 29)	anneals to bp 122-142 of the <i>pap</i> H gene of pHUR849, <i>pap</i> -5
REVSEQ	5' AAACACCATGAAACACACA 3' (SEQ ID NO: 30)	anneals to bp 41-61 of the <i>pap</i> H gene of pHUR849

^a contains a single Bam HI restriction site single underlined.

^b contains a single Sma I blunt end restriction site double underlined.

Please delete the paragraph on page 22, line 5 to page 23, line 6 and replace it with the following paragraph:

Nucleotide Sequences and Deduced *PapH* Primary Structures

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The plasmids pHUR849 (*pap-5*), pDAL201B (*pap-21*), pDAL210B (*pap-17*) and, pDAL200A (*pap-200A*), in *E. coli* strain HB101 express digalactose-binding of the serotypes F13, F7₁, F7₂ and F9, respectively. The *pap* gene cluster responsible for regulation and biogenesis of these pili from *E. coli* strains J96, C1212 and, 3669 is 1U. diagrammed in FIG. 1. Sequence analysis of *papH* genes from pDAL201B (*pap-21*), pDAL210B (*pap-17*) and, pDAL200A (*pap-200A*), was compared to the known nucleotide sequence of *papH* gene of pHUR849 (*pap-5*) (3). FIG. 2 shows a single 588-bp open reading frame with the same polarity as *papA* (2, 4). Analyses of these *papH* sequences revealed many typical features of prokaryotic gene organization. All four *papH* gene sequences contained a potential ribosome-binding sites, ATG initiation codon signal sequence, and a TGA termination codon. A potential initiation codon ATG at position -22, preceded by a sequence corresponding to -AGGGT, which showed homology to ribosome-binding sites, was found 13-bp upstream in all four *papH* sequences. A protein initiated here and ending at the TGA triplet at position 586 would encode a 195 amino acid polypeptide with a calculated molecular weight of 21.9 kd. The mature *PapH* protein contains 173 amino acid residues. The NH₂-terminal amino acid sequence of the open reading frame has all the features of a signal peptide sequence. The deduced putative signal sequence for the *papH* was located 22 codons upstream of their terminal Ala (FIG. 2). These sequences contained a highly hydrophobic region comprising an amino acids stretch of Ser-Val-Pro-Leu-Phe-Phe-Phe (residues -17 to -11 of SEQ ID NO: 32). There was a positively charge amino acid residue (Arg) at the position -21. The suggested cleavage sites between Ala -1 and gly +1 conforms to rules of prokaryotic signal cleavage sites and was similar to most other bacterial genes (12). In addition, the final *papH* deletion derivatives, pKD849-5 (*pap-5*), pKD201B (*pap-21*), pKD210B-1 (*pap-17*) and pKD200A-8 (*pap-200A*), were also sequenced. In addition, sequencing into the *papA* and *papC* genes which flank the *papH* gene (FIG. 1) of all four *papH* deletion derivatives was carried out in order to insure that all three genes were in frame. Finally, the codon usage of the *papH* genes of pDAL201B, pDAL210B and, pDAL200A, and *papH* gene of pHUR849 were analyzed using a codon frequency computer program (13). The pattern of codon utilization was not significantly different among the genes.